Effects of aluminium on the hepatic inositol polyphosphate phosphatase

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There is speculation that some of the toxic effects of Al^{3+} may originate from it perturbing inositol phosphate/ Ca^{2+} signalling. For example, in permeabilized L1210 mouse lymphoma cells, 10–50 μM Al^{3+} activated $\text{Ins}(1,3,4,5)\text{P}_4$ -dependent Ca^{2+} mobilization and $\text{Ins}(1,3,4,5)\text{P}_4$ 3-phosphatase activity [Loomis-Husselbee, Cullen, Irvine and Dawson (1991) *Biochem. J.* 277, 883–885]. $\text{Ins}(1,3,4,5)\text{P}_4$ 3-phosphatase activity is performed by a multiple inositol polyphosphate phosphatase (MIPP) that also attacks $\text{Ins}(1,3,4,5,6)\text{P}_5$ and InsP_6 [Craxton, Ali and Shears (1995) *Biochem. J.* 305, 491–498]: 5–50 μM Al^{3+} increased MIPP activity towards both $\text{Ins}(1,3,4,5)\text{P}_4$ (by 30%) and $\text{Ins}(1,3,4,5,6)\text{P}_5$ (by up to 500%), without affecting metabolism of InsP_6 . Higher concentrations of Al^{3+} inhibited metabolism of all three sub-

strates, and in the case of InsP_6 , Al^{3+} altered the pattern of accumulating products. When 1–50 μM Al^{3+} was present, InsP_6 became a less effective inhibitor of $\text{Ins}(1,3,4,5)\text{P}_4$ 3-phosphatase activity; this effect did not depend on the presence of cellular membranes, contrary to a previous proposal. The latter phenomenon largely explains how, in a cell-free system where $\text{Ins}(1,3,4,5)\text{P}_4$ 3-phosphatase is inhibited by endogenous InsP_6 , the addition of Al^{3+} can apparently increase the enzyme activity. However, there was no effect of either 10 or 25 μM Al^{3+} (in either the presence or absence of apotransferrin) on inositol phosphate profiles in either Jurkat E6-1 lymphoma cells or AR4-2J pancreatoma cells.

INTRODUCTION

A prediction that the vicinal 4,5-phosphates of $\text{Ins}(1,4,5)\text{P}_3$ might bind Al^{3+} avidly led to the proposal that both the actions of inositol phosphates and their metabolism might be particularly vulnerable targets for perturbation by Al^{3+} [1]. Subsequent reports that Al^{3+} disrupted Ca^{2+} -oscillatory activity in cells [2,3] reinforced the idea that some toxic effects of Al^{3+} may arise from interactions with the phosphoinositide signalling system. However, up to 40 μM Al^{3+} has since been shown not to affect either $\text{Ins}(1,4,5)\text{P}_3$ -mediated Ca^{2+} release or $\text{Ins}(1,4,5)\text{P}_3$ metabolism by the 5-phosphatase or 3-kinase [4]. On the other hand, the addition of Al^{3+} to permeabilized L1210 cells stimulated 3-phosphatase activity towards $\text{Ins}(1,3,4,5)\text{P}_4$, and the $\text{Ins}(1,4,5)\text{P}_3$ that was formed then mobilized intracellular Ca^{2+} stores [5]. These results suggested that 3-phosphatase activity might be a metabolic target during Al^{3+} intoxication *in vivo*. If so, it could provide a mechanism by which Al^{3+} might perturb cytosolic levels of $\text{Ins}(1,4,5)\text{P}_3$, $\text{Ins}(1,3,4,5)\text{P}_4$ and Ca^{2+} , as well as affecting the complex interactions between these cellular signals [6]. $\text{Ins}(1,3,4,5)\text{P}_4$ 3-phosphatase activity is performed by a multiple inositol polyphosphate phosphatase (MIPP) that also attacks $\text{Ins}(1,3,4,5,6)\text{P}_5$ and InsP_6 [7]; all three substrates are therefore potential targets of Al^{3+} . Another possibility relates to whether Al^{3+} might increase 3-phosphatase activity directly or indirectly through an interaction of Al^{3+} with other cellular constituents such as InsP_6 [5]. These uncertainties have prompted us to investigate the interactions of Al^{3+} with 3-phosphatase in some detail, and we have given attention to their possible physiological significance.

MATERIALS AND METHODS

Hepatic MIPP was purified as described previously [7]. Enzyme activity against 5 μM $\text{Ins}(1,3,4,5)\text{P}_4$, 1 μM $\text{Ins}(1,3,4,5,6)\text{P}_5$ or 0.05 μM InsP_6 was assayed at 37 °C in 100 μl of medium containing 100 mM KCl, 10 mM Hepes (pH 7.0), 1 mM dithio-

threitol, 3 mM MgCl_2 , 2.5 mM ATP, 10 $\mu\text{g/ml}$ creatine kinase, 2 mM CHAPS, 10 mM phosphocreatine, 50 μM GTP, 5 mM succinate (Na^+ salt) and 0.05% (w/v) BSA. The concentration of enzyme and other additions or modifications to the medium are provided in the Figure legends. Reactions to be assayed on gravity-fed columns were quenched with 1 ml of ice-cold medium containing 0.2 M ammonium formate, 0.1 M formic acid and 1 mg/ml InsP_6 . Samples were placed on ice for at least 5 min, protein precipitates were removed by brief centrifugation at 10000 g, and then samples were analysed on gravity-fed ion-exchange columns [7]. Some reactions were analysed by h.p.l.c. [8], and in these cases, they were first quenched with perchloric acid and neutralized with freon/octylamine [7]. Radioactivity in the h.p.l.c. eluate was measured on-line [8]. All enzyme assays were performed in triplicate, and the values of the resultant standard errors were less than 5% of the mean value.

Hepatic microsomes were also used as a source of MIPP [9]. AR4-2J cells were cultured as described in ref. [10]. Jurkat E6-1 cells (Advanced Biotechnologies) were cultured in RPMI 1640 medium with 10% fetal calf serum and 50 $\mu\text{g/ml}$ gentamicin. Cells were labelled with 50–100 $\mu\text{Ci/ml}$ [^3H]inositol for 96 h. Medium was then removed and replaced with Hepes-buffered Krebs–Ringer (pH 7.5) without added [^3H]inositol. Cells were then incubated for up to 2 h at 37 °C with 0, 10 or 25 μM AlCl_3 , in the presence or absence of 10 μM apotransferrin. In alternative experiments, Al^{3+} was present for the last 24 h of the [^3H]inositol labelling procedure. In the experiments with AR4-2J cells, 0, 10 or 25 μM AlCl_3 (in the presence or absence of 10 μM apotransferrin) was added for the last 20 h of the [^3H]inositol labelling procedure. Incubations containing either AR4-2J cells or Jurkat E6-1 cells were quenched with perchloric acid, neutralized with freon/octylamine and analysed on either an Adsorbosphere SAX h.p.l.c. column or a Partisphere SAX h.p.l.c. column as described previously [8,10].

AlCl_3 was purchased from Aldrich; solutions containing Al^{3+} were made freshly with deionized water and were kept in plastic

Abbreviation used: MIPP, multiple inositol polyphosphate phosphatase [7].

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containers. Sodium succinate and GTP were purchased from Sigma. Apotransferrin (product number 1317393) was obtained from Boehringer-Mannheim. Sources of other materials are as given previously [7–10]. The OKT3 antibody was obtained from Division of Cancer Treatment, National Cancer Institute, MD, U.S.A.

RESULTS AND DISCUSSION

Effects of Al^{3+} on $\text{Ins}(1,3,4,5)\text{P}_4$ 3-phosphatase activity of MIPP

In a previous study with permeabilized L1210 cells [5], the addition of 10–200 μM Al^{3+} progressively increased the amount of Ca^{2+} released on addition of $\text{Ins}(1,3,4,5)\text{P}_4$. Loomis-Husselbee et al. [5] postulated that this effect of Al^{3+} involved activation of an $\text{Ins}(1,3,4,5)\text{P}_4$ 3-phosphatase, with the ensuing generation of $\text{Ins}(1,4,5)\text{P}_3$ being largely responsible for the Ca^{2+} release. Indeed, these workers further showed that 50 μM Al^{3+} caused a 2-fold activation of $\text{Ins}(1,3,4,5)\text{P}_4$ 3-phosphatase activity in the permeabilized cells.

Loomis-Husselbee et al. [5] suggested that Al^{3+} might activate the 3-phosphatase either directly or indirectly through an interaction with endogenous inhibitors of this enzyme. In order to distinguish between these two alternative hypotheses, we first investigated the effect of Al^{3+} on the $\text{Ins}(1,3,4,5)\text{P}_4$ 3-phosphatase activity of purified MIPP, which is free of endogenous inhibitors [7]. We incubated the enzyme in the 'intracellular' medium used by Loomis-Husselbee et al. [5]. Al^{3+} inhibited enzyme activity, with an $\text{IC}_{50} > 150 \mu\text{M}$ (Figure 1). Despite this effect, lower concentrations of Al^{3+} slightly activated the enzyme (Figure 1): in four experiments (including that described in Figure 1), both 5 and 10 μM Al^{3+} consistently activated the rate of $\text{Ins}(1,3,4,5)\text{P}_4$ hydrolysis by $26 \pm 5\%$ and $33 \pm 10\%$ respectively. However, the extent of this effect was by itself insufficient to account for the Al^{3+} -mediated 2-fold increase in accumulation of $\text{Ins}(1,4,5)\text{P}_3$ during $\text{Ins}(1,3,4,5)\text{P}_4$ metabolism by permeabilized L1210 cells [5].

One mode of perturbation of enzymes by Al^{3+} is by competition with Mg^{2+} [1]. Indeed, Figure 1 provides some evidence that Mg^{2+} protects against Al^{3+} -dependent inhibition of 3-phosphatase: in these experiments, omission of Mg^{2+} from the incubation

Figure 1 Effect of Al^{3+} on $\text{Ins}(1,3,4,5)\text{P}_4$ 3-phosphatase activity of MIPP

Purified MIPP was incubated with the indicated concentration of Al^{3+} for 20 min at 37 °C at a concentration of 0.01–0.04 μg of protein/ml in a volume of 100 μl with 5 μM [^3H] $\text{Ins}(1,3,4,5)\text{P}_4$ in the medium described in the Materials and methods section (●). Additional experiments were performed in the absence of Mg^{2+} (▼). Data represent means of triplicate determinations from one of four similar experiments. Three further experiments were performed using microsomes as the source of MIPP [9], and similar results were obtained (in the latter case, reactions were analysed by h.p.l.c. [8] to distinguish the products of 3-phosphatase activity from some contaminating $\text{Ins}(1,3,4,5)\text{P}_4$ 5-phosphatase).

Figure 2 Effects of InsP_6 and Al^{3+} on $\text{Ins}(1,3,4,5)\text{P}_4$ 3-phosphatase activity of MIPP

Purified MIPP was incubated with the indicated concentration of Al^{3+} as described in the legend to Figure 1, except that one of the following concentrations of InsP_6 was also added to the incubations: 0 (●), 50 (▲) or 100 (■) nM.

Figure 3 Inhibition of InsP_6 dephosphorylation by Al^{3+}

Microsomes were incubated with the indicated concentrations of AlCl_3 for 30 min at 37 °C in a volume of 100 μl with 50 nM [^3H] InsP_6 in the medium described in the Materials and methods section. Reactions were quenched with perchloric acid, neutralized and analysed by h.p.l.c. on an Adsorbosphere 5 μM SAX column [8]. Data are from one of four similar experiments. Similar results were obtained in three further experiments in which the purified MIPP was used.

medium resulted in Al^{3+} becoming a more potent inhibitor of $\text{Ins}(1,3,4,5)\text{P}_4$ 3-phosphatase activity (IC_{50} 50 μM ; Figure 1).

Interactive effects of Al^{3+} and InsP_6 on 3-phosphatase

Cells contain potent endogenous inhibitors of $\text{Ins}(1,3,4,5)\text{P}_4$ 3-phosphatase activity, such as InsP_6 [7,9,11]. Loomis-Husselbee et al. [5] suggested that if Al^{3+} were able to neutralize the effects of such endogenous inhibitors, then this might provide a mechanism by which this cation could increase apparent $\text{Ins}(1,3,4,5)\text{P}_4$ 3-phosphatase activity. We therefore assessed the effects of Al^{3+} on the $\text{Ins}(1,3,4,5)\text{P}_4$ 3-phosphatase activity of purified MIPP when it was also incubated with exogenous inhibitor (i.e. InsP_6). We found that the metabolism of 5 μM $\text{Ins}(1,3,4,5)\text{P}_4$ was 72% and 86% inhibited by 50 and 100 nM InsP_6 respectively (Figure 2). Moreover, the further addition of 1–50 μM Al^{3+} led to a dose-dependent reversal of this inhibition. In other words, in the presence of InsP_6 , Al^{3+} activated $\text{Ins}(1,3,4,5)\text{P}_4$ 3-phosphatase activity by up to 4-fold (Figure 2). These results can account for

Figure 4 Effect of Al^{3+} on the specificity of InsP_6 phosphatase activity of MIPP

Purified MIPP (0.5–2 μg of protein/ml) was incubated with $[\text{³H}]\text{InsP}_6$ in the absence (a) or presence (b) of 200 μM Al^{3+} as described in the legend to Figure 2. Reactions were quenched with perchloric acid, neutralized and analysed on an Adsorbosphere SAX column [8]; radioactivity in the eluate was measured on-line [8]. Information cited in [8] identifies the constituents of the three InsP_5 peaks. The integrated c.p.m. for the various peaks (in order of elution) were as follows: (a) InsP_3 = 235 c.p.m.; InsP_4 = 949 c.p.m.; $\text{Ins}(1,2,3,4,6)\text{P}_5$ = 214 c.p.m.; $\text{D/L-Ins}(1,2,3,4,5)\text{P}_5$ = 415 c.p.m.; $\text{S/L-Ins}(1,2,4,5,6)\text{P}_5 + \text{Ins}(1,3,4,5,6)\text{P}_5$ = 690 c.p.m.; InsP_6 = 2910 c.p.m. (b) InsP_3 = 170 c.p.m.; InsP_4 = 832 c.p.m.; $\text{Ins}(1,2,3,4,6)\text{P}_5$ = 1009 c.p.m.; $\text{D/L-Ins}(1,2,3,4,5)\text{P}_5$ = 166 c.p.m.; $\text{D/L-Ins}(1,2,4,5,6)\text{P}_5 + \text{Ins}(1,3,4,5,6)\text{P}_5$ = 119 c.p.m.; InsP_6 = 3816 c.p.m. Data are representative of five experiments (two with the purified enzyme and three with microsomes).

the activation of $\text{Ins}(1,3,4,5)\text{P}_4$ 3-phosphatase activity by Al^{3+} in permeabilized L1210 cells [5], provided we make the reasonable assumption that these cells retained some endogenous InsP_6 .

Loomis-Husselbee et al. [5] also suggested that if Al^{3+} were able to reverse inhibition of $\text{Ins}(1,3,4,5)\text{P}_4$ 3-phosphatase by InsP_6 , this effect might be promoted by cell membranes, which can bind substantial amounts of InsP_6 in an Al^{3+} -dependent manner [12]. Although we cannot exclude this possibility, we have shown that Al^{3+} can neutralize the effects of InsP_6 on the purified enzyme (Figure 2), and as such this effect does not depend on the presence of cell membranes.

Since an interaction of Al^{3+} with InsP_6 reduced the latter's efficacy as an inhibitor of 3-phosphatase activity, we investigated whether Al^{3+} could affect InsP_6 hydrolysis itself. We found that Al^{3+} inhibited InsP_6 metabolism, albeit with slightly less potency (IC_{50} approx. 200 μM ; Figure 3) than Al^{3+} inhibited $\text{Ins}(1,3,4,5)\text{P}_4$ metabolism (IC_{50} approx. 150 μM ; Figure 1). InsP_6 hydrolysis by this enzyme normally yields the entire spectrum of InsP_5 isomers, although $\text{Ins}(1,2,3,4,6)\text{P}_5$ is a minor product (Figure 4). However, concentrations of Al^{3+} that inhibited InsP_6 hydrolysis (i.e. > 100 μM) also altered the pattern of accumulating products so that $\text{Ins}(1,2,3,4,6)\text{P}_5$ became the major InsP_5 isomer to accumulate (Figure 4). Perhaps Al^{3+} causes the enzyme to have a greater preference for the 5-phosphate of InsP_6 because this position is furthest from the

Figure 5 Effect of Al^{3+} on $\text{Ins}(1,3,4,5,6)\text{P}_6$ 3-phosphatase activity of MIPP

Purified MIPP was incubated for 30 min at 37 °C at a concentration of 0.1–0.2 μg of protein/ml in a volume of 100 μl with 1 μM $[\text{³H}]\text{Ins}(1,3,4,5,6)\text{P}_6$ in the media described in the legend to Figure 1 [+ Mg^{2+} (●); – Mg^{2+} (▼)] plus the concentrations of AlCl_3 indicated in the Figure. Reactions were quenched as described in the legend to Figure 1, and mixtures analysed on gravity-fed anion-exchange columns. Data are from one of two similar experiments; two additional experiments with microsomes yielded similar results

1,2,3-phosphate cluster which may be where Al^{3+} preferentially complexes to InsP_6 [12].

Effects of Al^{3+} on InsP_6 hydrolysis by MIPP

The experiments described above demonstrate that Al^{3+} could slightly (approx. 30 %) activate $\text{Ins}(1,3,4,5)\text{P}_4$ 3-phosphatase activity (Figure 1). We have also found that hydrolysis of $\text{Ins}(1,3,4,5,6)\text{P}_5$ was activated by Al^{3+} , although much more dramatically: 5–150 μM Al^{3+} increased $\text{Ins}(1,3,4,5,6)\text{P}_5$ 3-phosphatase activity by up to 5-fold (Figure 5). We demonstrated that the resultant InsP_4 product was $\text{Ins}(1,4,5,6)\text{P}_4$ (i.e. the same InsP_4 that is formed in the absence of Al^{3+} [7]) since it was a substrate for the partially purified $\text{Ins}(1,4,5,6)\text{P}_4$ 3-kinase [13] (results not shown). Thus Al^{3+} did not alter the specificity of the enzyme towards the 3-phosphate of $\text{Ins}(1,3,4,5,6)\text{P}_5$, even though it changed the products formed when this phosphatase attacked InsP_6 (Figure 4). When Mg^{2+} was omitted from the incubation buffer, the resultant stimulatory and inhibitory effects of Al^{3+} were, in general, more acute (Figure 5).

Can Al^{3+} affect inositol phosphate turnover in intact cells?

The activation of $\text{Ins}(1,3,4,5,6)\text{P}_6$ hydrolysis by as little as 5 μM Al^{3+} (Figure 5) is the most potent, direct effect of this cation on inositol phosphate metabolism that we have observed. It was important to consider whether Al^{3+} might have an effect on metabolism of $\text{Ins}(1,3,4,5,6)\text{P}_6$ or other inositol polyphosphates *in vivo*. We therefore investigated the effect of Al^{3+} on inositol phosphate profiles in two types of intact cultured cell lines: the AR4-2J pancreatoma and the Jurkat E6-1 lymphoma. These choices reflected the possibility that these particular cells might offer 3-phosphatase as a particularly accessible target for Al^{3+} . For example, AR4-2J cells contain unusually high levels of $\text{Ins}(1,4,5,6)\text{P}_4$ [10], which suggests an atypical expression of $\text{Ins}(1,3,4,5,6)\text{P}_5$ 3-phosphatase activity. The Jurkat cells were chosen because their receptor-activated $\text{Ins}(1,3,4,5)\text{P}_4$ signal is particularly striking [14] (also see below) and also because a report of high levels of $\text{D/L-Ins}(1,2,4,5,6)\text{P}_5$ in these cells (around 40 % of the levels of InsP_6) is consistent with relatively high inherent catabolism of InsP_6 [14]. However, in our Jurkat cells, the level of $\text{D/L-Ins}(1,2,4,5,6)\text{P}_5$ was only 3 % of that of

Figure 6 Lack of effect of Al^{3+} on $[^3\text{H}]$ inositol phosphates in $[^3\text{H}]$ inositol-labelled Jurkat cells

Jurkat cells were labelled with $[^3\text{H}]$ inositol as described in the Materials and methods section, and during the last 24 h, $10 \mu\text{M}$ apotransferrin was added plus either 0 or $10 \mu\text{M}$ AlCl_3 . Cells were subsequently treated with $10 \mu\text{g/ml}$ OKT3 for 10 min. Incubation mixtures were quenched with perchloric acid, neutralized and chromatographed on an Adsorbosphere 5 μM SAX column. The following data are the integrated c.p.m. for each peak (identified as in [8]); these are given in pairs, first for $0 \mu\text{M}$ Al^{3+} ($\circ$) followed by the data for $10 \mu\text{M}$ Al^{3+} ($\bullet$). Peak A, not identified = 1421/1423; peak B, $\text{Ins}(1,3,4)\text{P}_3$ = 642/709; peak C, $\text{Ins}(1,4,5)\text{P}_3$ = 1023/1037; peak D, $\text{Ins}(1,3,4,6)\text{P}_4$ = 385/385; peak E, $\text{Ins}(1,3,4,5)\text{P}_4$ = 2304/2030; peak F, $\text{Ins}(3,4,5,6)\text{P}_4 + \text{Ins}(1,4,5,6)\text{P}_4$ = 459/393; peak G, total InsP_5 = 2645/2613; peak I, InsP_6 = 11 082/10 946. Inset: similar samples were chromatographed on a Partisphere SAX column and 1 min fractions were collected. The following data are the d.p.m./fraction for each peak, and in each case these are given in pairs, first for $0 \mu\text{M}$ Al^{3+} ($\circ$) followed by the data for $10 \mu\text{M}$ Al^{3+} ($\bullet$): peak G, $\text{Ins}(1,3,4,5,6)\text{P}_5$ = 9004/8544; peak H, $\text{D/L-Ins}(1,2,4,5,6)\text{P}_5$ = 777/646; peak I, InsP_6 = 37175/34392.

$[^3\text{H}]\text{InsP}_6$ (inset to Figure 6) which is similar to that usually found in other cell types [8]. $\text{D/L-Ins}(1,2,4,5,6)\text{P}_5$ incorporates $[^3\text{H}]$ inositol at the same rate as InsP_6 [8] (results not shown), so the difference between the two studies is unlikely to reflect inadequate labelling of InsP_6 in our cells. Nevertheless, it is an important point to note that we did not have a good intact cell model for studying the effect of Al^{3+} on the metabolism of $\text{Ins}(1,2,4,5,6)\text{P}_5$, which is a quantitatively important and receptor-regulated polyphosphate in at least one other T-lymphocyte cell line [14].

Normal serum Al^{3+} levels are below $1 \mu\text{M}$ [15], with levels of $5\text{--}10 \mu\text{M}$ being associated with Al^{3+} toxicity [16]. We therefore incubated intact cells with 0, 10 or $25 \mu\text{M}$ Al^{3+} . In addition, we performed experiments in the presence of $10 \mu\text{M}$ apotransferrin to increase cellular accumulation of Al^{3+} [17]. Data from a representative experiment with OKT3-stimulated Jurkat cells are shown in Figure 6, in which the receptor-dependent increase in $\text{Ins}(1,3,4,5)\text{P}_4$ levels was 30-fold; Al^{3+} did not alter the profile of intracellular inositol phosphates. Indeed, the absence of an effect of Al^{3+} on phospholipase C activity, which is critically dependent on cellular ATP for a continuous supply of lipid substrate, is more generally indicative of these levels of Al^{3+} not being cytotoxic. Al^{3+} also did not affect inositol phosphate profiles of unstimulated Jurkat cells or AR4-2J cells (results not shown).

Concluding comments

We have shown that Al^{3+} has a variety of dramatic and complex effects on isolated 3-phosphatase activity. Some of these observations provide the first direct explanation for the observation [5] that low concentrations of Al^{3+} can apparently promote $\text{Ins}(1,3,4,5)\text{P}_4$ 3-phosphatase activity *in vitro*. We have uncovered two contributory factors: first, $5\text{--}10 \mu\text{M}$ Al^{3+} itself directly activates $\text{Ins}(1,3,4,5)\text{P}_4$ 3-phosphatase activity of MIPP by approximately 25–30% (Figure 1). Second, and quantitatively more importantly, Al^{3+} can act indirectly by partially reversing inhibition of $\text{Ins}(1,3,4,5)\text{P}_4$ 3-phosphatase activity by InsP_6 (Figure 2). The first of these two effects may involve interaction of Al^{3+} with the site at which Mg^{2+} acts to modify 3-phosphatase activity [11], since these two cations can be competing ligands [1]. It is harder to understand the mechanism that underlies the reversal, by Al^{3+} , of InsP_6 -dependent inhibition of $\text{Ins}(1,3,4,5)\text{P}_4$ 3-phosphatase; Al^{3+} binds tightly to InsP_6 [12], and the resultant complex may then have a reduced affinity to compete with $\text{Ins}(1,3,4,5)\text{P}_4$ for access to the enzyme's active site. However, this seems unlikely, because up to $150 \mu\text{M}$ Al^{3+} had no significant effect on InsP_6 hydrolysis itself (Figure 3). The idea [5] that cell membranes may be necessary for Al^{3+} to prevent InsP_6 from inhibiting 3-phosphatase now seems unlikely, as these effects can be observed with the purified enzyme.

A major difficulty that impedes our understanding of the biochemical basis for Al^{3+} toxicity relates to the uncertain relevance of a number of *in vitro* models. It is typical of this controversy that there are conflicting data relating to the interactions of Al^{3+} with inositol phosphate/ Ca^{2+} signalling systems. Our studies with purified MIPP confirm and extend a study with permeabilized cells [5] concerning the considerable effects on this enzyme of pathologically relevant concentrations of Al^{3+} . However, we have not found any evidence to indicate that any of these phenomena occur in intact cells treated with similar amounts of Al^{3+} . This conclusion mirrors that of earlier studies of cellular $[^3\text{H}]\text{InsP}_3$, in which it required Al^{3+} at a concentration higher than $100 \mu\text{M}$ to be added to intact cells before levels of total $[^3\text{H}]\text{InsP}_3$ decreased [18–20]. In contrast, in permeabilized cells, as little as $5\text{--}15 \mu\text{M}$ Al^{3+} can inhibit phospholipase C and thereby inhibit the release of $\text{Ins}(1,4,5)\text{P}_3$ [18,21]. These empirical differences highlight the importance of the goal of defining appropriate cell preparations for obtaining physiologically relevant information on the effects of Al^{3+} on inositol phosphate/ Ca^{2+} signalling.

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